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HSM-LUP-GYR

European Patent Office
Gitschiner Str. 103
D-10969 Berlin
Germany
(Tel: +49 30 25901-840)

3 November 2004

BY COURIER

Dear Sirs,

Re: LUPIN LTD
PCT International Application
No. PCT/IN 02/00192
Filed on: 20/09/2002
Response to the Second Written Opinion
Due date 9th November 2004

We respectfully refer to Written Opinion mailed on 25th August 2004. The due date for response is 9th November 2004.

A response to the Written Opinion is enclosed herewith. The response comprises:

1. A detailed reply regarding novelty and inventiveness of the present invention.
2. Clean copies of amended page 13
3. Comparison document

It is respectfully submitted that the detailed response submitted herewith clearly establishes novelty and inventiveness.

It is respectfully requested that the response is duly considered and a favourable International Preliminary Examination Report is established.

Yours truly,

Hariharan Subramaniam

EXPRESS MAIL LABEL
NO.: EV 481673195 US

PCT/IN 02/00192

Applicants' file reference: HSM-LUP-GYR

European Patent Office
Gitschiner Str. 103
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PCT International Application
PCT/IN02/00192

§ HSM-LUP-GYR No.
§
§
§ Authorised Officer: Mr. Fuchs, U
Applicant: Lupin Limited §
§
§

International Filing date: 20 September 2002
Priority date: 20 September 2002

Enclosed with this response are the following:

1. A detailed reply regarding novelty and inventiveness of the present invention.
2. Clean copies of amended page 13
3. Comparison document (page 13)

The Search Report cites several documents of which only D1, i.e., XP002242358 (U. H. Manjunatha *et al.*) is an **X** category citation. All other citations are only **A** category citations. Since these do not affect the novelty and inventiveness of the applicants' invention, only U. H. Manjunatha *et al.* is addressed herewith.

U. H. Manjunatha et.al., "Monoclonal antibodies to mycobacterial DNA gyrase A inhibit DNA supercoiling activity",*Eur. J. Bicochem.*, 200, 268, 2038-2046 (XP-00224358), (hereinafter referred to as D1)

Without prejudice to what was said in our response dated 26 July 2004, we respectfully submit as follows:

Revised description submitted along with revised claims in response to the first written opinion, pertains to engineered single chain antibody that inhibits DNA gyrase from M. *smegmatis* and M. *tuberculosis*.

Chains 1 to 6 and 8 describe engineered single chain antibodies nucleotide sequence and polypeptide sequence of the recombinant SCFV gene, peptide sequences thereof and the process of preparation thereof.

Claim 7 describes monoclonal antibodies, which inhibit DNA gyrase from fluoroquinolone resistant M. *smegmatis* and M. *tuberculosis*. **It does not describe the inhibition of the wild type enzyme described in U.H. Manjunatha** *et al.* Further, U.H. Manjunatha *et al.* describes Mabs that bind to DNA gyrase from various mycobacterial species and also inhibit DNA gyrase from M. *smegmatis*. This publication does not show any effect of MSGyrA:C3/MSGyrA:H11 Mabs on M. *tuberculosis* gyrase activity. Nor does it show any data on drug resistant fluoroquinolone resistant M. *tuberculosis*/ M. *smegmatis* DNA gyrase activities which is now shown (claim 7). Fluoroquinolones are potent DNA gyrase inhibitors and the resistance to them means altered enzyme structure. It is well known that antibiotic resistance could alter the structural features of the proteins/enzymes. These data imply that the Mabs have different mechanism of action as enzymes do not show cross resistance to them.

This inhibition property described in the application is not disclosed before. Thus the citation D1 does not teach any of the features of amended claim 7 or render the features of Claim 7 obvious.

In view of the submissions presented above and the clarificatory amendments made to the specification and claims in response to the first written opinion, it is respectfully submitted that the claims are novel and inventive over the prior art.

Accordingly, we respectfully request that a favourable International Preliminary Examination Report be issued.

Yours faithfully,

Hari. Subramaniam

Enclosures
Corrected page 13
Comparison page 13 showing amendments

inhibited at 3 μg/ml and 6 μg/ml concentrations of MsGyrA:C3 for quinolone sensitive (D^S) and quinolone resistant (D^R) enzymes respectively (Figure 3B). The twofold difference in the mAb concentration between D^S and D^R enzymes is attributed to reduced specific activity of D^R enzyme. DNA gyrase from ofloxacin resistant, highly virulent clinical isolate of *M. tuberculosis* (ICC-222) was also assayed for the effect of mAb. The purified enzyme has an IC_{50} of ~10 μg/ml for ciprofloxacin, where as the MsGyrA:C3 inhibited DNA gyrase supercoiling activity at 3.0 μg/ml, similar to that of *M. smegmatis* enzyme (Figure 3C). The absence of cross-resistance essentially emphasizes the mode of action of mAb to be distinct to that of quinolones. Similar to MsGyrA:C3, MsGyrA:H11 also inhibited ciprofloxacin resistant *M. smegmatis* DNA gyrase (Figure 3D). These data confirm the novel inhibition mechanism of gyrase by mAb. Absence of cross-resistance to fluoroquinolone resistant DNA gyrase by mAb, warrants the study of MsGyrA:C3 further as it could aid in countering the drug resistance problem.

E. Cloning, sequencing and expression of a DNA sequence encoding for neutralizing antibody gene and design of bioactive peptides

This example describes the cloning and expression of a nucleic acid sequence coding for a DNA gyrase neutralizing ~~monoclonal~~ single chain antibody, scFv:GyrA.. Based on the inhibition of gyrase by scFv:GyrA and utilizing sequence of the antibody, bioactive peptides were designed and their inhibition of mycobacterial DNA gyrase was tested.

E1 : ***Cell culture and Isolation of RNA:***

Total RNA was isolated from the actively secreting mAb:C3 hybridoma cell line. Briefly, confluent hybridoma cells ($3X10^8$) were washed with ice cold IMDM medium and total RNA was extracted using TRIzol reagent (Life technologies Inc). RNA was purified using RNeasy QUIAGEN as per the manufacturer's protocol. The quality of RNA was confirmed by electrophoresis in a 1% formaldehyde agarose gel.

E2 : ***First-strand cDNA synthesis:***

The first-strand cDNA was synthesized from total RNA using the reverse transcription reaction (RT). For annealing, 5 μg of total RNA was incubated with 0.2 μg/ml of random hexamer oligonucleotide in a 10 μl reaction volume at 70°C for 5 minutes, followed by immediate chilling on ice. The annealed mix was incubated with 1 mM dNTP and 20 Units of Moloney Murine Leukemia Virus reverse transcriptase, (M-

inhibited at 3 μg/ml and 6 μg/ml concentrations of MsGyrA:C3 for quinolone sensitive (D^S) and quinolone resistant (D^R) enzymes respectively (Figure 3B). The twofold difference in the mAb concentration between D^S and D^R enzymes is attributed to reduced specific activity of D^R enzyme. DNA gyrase from ofloxacin resistant, highly virulent clinical isolate of *M. tuberculosis* (ICC-222) was also assayed for the effect of mAb. The purified enzyme has an IC_{50} of ~10 μg/ml for ciprofloxacin, where as the MsGyrA:C3 inhibited DNA gyrase supercoiling activity at 3.0 μg/ml, similar to that of *M. smegmatis* enzyme (Figure 3C). The absence of cross-resistance essentially emphasizes the mode of action of mAb to be distinct to that of quinolones. Similar to MsGyrA:C3, MsGyrA:H11 also inhibited ciprofloxacin resistant *M. smegmatis* DNA gyrase (Figure 3D). These data confirm the novel inhibition mechanism of gyrase by mAb. Absence of cross-resistance to fluoroquinolone resistant DNA gyrase by mAb, warrants the study of MsGyrA:C3 further as it could aid in countering the drug resistance problem.

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